

Localized and Systemic Hypersensitivity Reactions to Human Seminal Fluid

I. LEONARD BERNSTEIN, M.D.; BRUCE E. ENGLANDER, M.D.; JOAN S. GALLAGHER, Ph.D.; PAUL NATHAN, Ph.D.; and ZVI H. MARCUS, Ph.D.; Cincinnati, Ohio

Reaginic humoral antibodies to human seminal plasma were present in two women with systemic reactions. In one patient, IgE antibodies were demonstrated by direct skin tests, leukocyte histamine release, passive transfer to a nonallergic human recipient, the radioallergosorbent test, inhibition of the radioallergosorbent test and neutralization of passive transfer antibodies. A similar mechanism was established in the other case by direct skin tests and antigen-induced leukocyte histamine release. Sephadex G-100 fraction 2, derived from human seminal plasma, showed greater reaginic activity than other chromatographic fractions. Two other patients with histories of localized reactions did not develop humoral antibodies but did evince cell-mediated immunity to seminal fluid antigens. One patient with localized reactions also had significant titers of IgM and IgG sperm agglutinating antibodies to seminal plasma. Both reaginic and cell-mediated sensitization to human sperm extract were also observed in this series of patients. Histocompatibility leukocyte antigen (HLA) typing of all patients and their sexual partners showed a marked degree of shared histocompatibility locus antigens in members of each of two couples.

IMMUNOLOGIC responses to seminal fluid antigens in women may be sequelae of local or systemic antibody formation, or both (1). Seminal antigens that are absorbed within the female reproductive tract may subsequently be phagocytosed by macrophages and finally, may stimulate isoimmunization after appropriate T—B cell interactions (1). The occurrence of systemic immune responses to seminal fluid is best illustrated by anaphylactic reactions which are mediated by IgE antibodies (2-11). Local immune responses to human spermatozoa and seminal fluid have also been described (12). Relatively little is known about cell-mediated mechanisms in this type of seminal fluid hypersensitivity. Anti-sperm IgG or IgM antibodies also may play a role in female sterility problems (13, 14).

We studied the genetic susceptibility, general immunologic status, and specific immunoreactivity to seminal fluid and spermatozoal antigens of four women who experienced severe post-coital reactions. Two patients showed symptomatology consistent with IgE-mediated hypersensitivity, and the other two developed localized adverse reactions with evidence of cell-mediated immune response.

Materials and Methods

PATIENTS

Four women with severe adverse reactions during and after coitus were evaluated. Patients 1 and 2 had experienced life-

threatening reactions. Patients 3 and 4 had developed local reactions: burning pain, erythema, and edema of genital tissues lasting from 24 to 48 hours. The pertinent clinical features in each of these cases are summarized in Table 1.

The systemic symptoms experienced by Patients 1 and 2 required prompt treatment with epinephrine. Both patients remained free of symptoms as long as condoms were used during intercourse. Patient 1 had her initial episode of anaphylaxis 6 weeks after delivery of a normal baby, 10 weeks after the last coitus during pregnancy. Patient 2 developed anaphylactic symptoms after her first sexual intercourse. Both patients with immediate hypersensitivity reactions were atopic by history and skin tests.

Patients 3 and 4 complained only of localized intravaginal, peri-vaginal and vulvar symptoms. Both noted severe pain when their partners ejaculated. This pain lasted for several hours until superseded by an intense redness and swelling for the next several hours. The inflammatory response reached peak intensity within 24 hours. These symptoms began 4 weeks after the first intercourse in patient 3 and at the time of first intercourse in patient 4. All patients except Patient 4 had positive family histories of atopy as well as one or more personal allergic diatheses.

ISOLATION OF SPERMATOZOA AND SEMINAL PLASMA

The in-vitro and in-vivo tests were performed primarily with fresh ejaculates of sexual partners. Seminal fluid for fractionation experiments was collected on 4 consecutive days, pooled, and stored at -4°C before transport to the laboratory. The ejaculates were allowed to liquify at room temperature for 30 to 60 min and an equal volume of 0.05 M phosphate buffer, pH 7.4, was added. Centrifugation was done at 4°C for 30 minutes at 1000 rpm. Supernatants containing the diluted seminal plasma were used as fresh reagents that day or stored at -70°C for subsequent in vitro testing. The pelleted human spermatozoa preparations were washed three times in phosphate buffers and brought to a concentration between 50 to 100×10^6 cells/mL. The suspension was pulse-sonicated (6-s intervals) at 4°C for 60-s using a Branson Sonifier Cell Disruptor, Model W185 (Heat Systems, Ultrasonics Inc., Plainview, New York). Glass beads were added to the sperm suspension to facilitate disruption. The sonicated sperm suspension was centrifuged at 4°C at 27 000 g for 1 hour. The supernatant, spermatozoa extract, was removed and used in the in-vitro studies.

SEPHADEX G-100, CHROMATOGRAPHY OF SEMINAL PLASMA

Seminal plasma was centrifuged for 60 minutes at 15 000 rpm and 4°C (15). The clear supernatant was adjusted to a final concentration of 10 mg protein/mL by an immiscible molecular separator (Millipore Corp., Bedford, Massachusetts). The seminal plasma was dialyzed for 24 hours against phosphate buffer at 4°C , and was layered onto a Pharmacia column containing Sephadex G-100 (Pharmacia Diagnostics, Piscataway, New Jersey) which had been previously equilibrated with phosphate buffer. Elution was done with phosphate buffer and 2 mL fractions were collected. The protein content of unfractionated seminal plasma as well as the various fractions were determined by the Folin method (16). Peak fractions were lyophilized, resuspended in a minimum of distilled water, and dialyzed against phosphate buffer at 4°C for 48 hours. Figure 1 shows a representative elution pattern derived from Sephadex G-100 chroma-

► From the Departments of Internal Medicine, Obstetrics and Gynecology, and Physiology; University of Cincinnati Medical Center; Cincinnati, Ohio.

Table 1. Clinical Features

Patient	Type of Reaction	Number of Episodes	Onset	Atopic Background
1	Systemic	2	6 wks after pregnancy	Positive
2	Systemic	6	Immediately after first coitus	Positive
3	Localized	Many	4 wks after first coitus	Negative
4	Localized	Many	Immediately after first coitus	Positive

tography. Five major protein peaks could be identified. All experimental procedures using fractions of seminal plasma were done with peaks 1, 2, 3 and 4. Fractions 4 and 5 were found to be biologically inert in all experiments and will not be discussed further.

IMMUNOLOGIC TESTS

Direct Skin Tests: Scratch tests were done with spermatozoa extract, unfractionated seminal plasma and seminal plasma. Intracutaneous tests were done by a skin threshold procedure, beginning with a dilution of 1×10^{-6} g/mL and progressing up to and including 1×10^2 g/mL. Immediate skin responses were interpreted within 20 minutes according to a previously described protocol (17). Only skin tests manifesting two plus or greater reactions (a wheal at least two times the size of the control) were considered positive.

Passive Transfer Tests: These studies were done on the back region of two normal volunteer males after obtaining their informed consent. Skin sites were prepared by 0.10 mL intracutaneous injections of varying dilutions of fresh, unheated sensitive sera. One site was prepared with undiluted serum heated at 56°C for 2 hours. Two additional sites were included in the neutralization experiments. One of these was serum diluted 1:2; the other was an equal mixture of undiluted test serum and seminal plasma (1 mg protein/mL). Forty-eight hours later, these sites were challenged with 0.02 mL of spermatozoa extract, seminal plasma and seminal plasma fractions at various dilutions ranging from 1:100 to 1:100 000 (weight/volume w/v). Additional control skin test sites included phosphate-buffered saline (pH 7.4) and all the above substances in areas of skin not previously injected with test sera. Crossed diameters of wheals and erythema were recorded at 20 minutes and respective areas calculated by the formula $A = \frac{\pi d^2}{4}$ where d equaled

the mean of long and perpendicular (short) diameters. Only wheal areas have been included in this report.

Passive Cutaneous Anaphylaxis: This test was used as a substitute for human passive transfer in Patient 3 because of the unavailability of a human volunteer recipient. Macaca irus monkeys were anesthetized with phencyclidine hydrochloride (Sernylan; Parke-Davis, Morris Plains, New Jersey). Various skin sites were sensitized with 0.10 mL aliquots of serum. Twenty-four hours later, 5 mL of 0.5% Evans blue plus 1 mL (1 mg protein/mL) of respective antigens (spermatozoa extract and seminal plasma) were injected into the saphenous vein. Crossed diameters of blue areas at reaction sites were measured at 5 minutes, 2 hours and 24 hours after injection of antigens.

Total and Specific IgE: Total IgE was determined by a commercial radioimmunosorbent assay (Pharmacia Laboratories, Piscataway, New Jersey). Specific IgE was measured by a modification of the radioallergosorbent test (18). Spermatozoa extract, unfractionated seminal plasma and concentrated fractions of seminal plasma were coupled to cyanogen bromide-activated methylcellulose discs at concentrations ranging from 20 to 70 mg protein/mL. After stepwise incubation of antigen coupled discs with patient's serum and ^{125}I anti-IgE, radioactivity bound

to the paper discs was measured in a gamma counter. The radioactivity bound in the patient's serum was compared to similar measurements obtained from pooled serum of three normal volunteers. Because of the unusual incidence of blood group A in three of four male sexual partners, a modified blood substance A-specific radioallergosorbent test was done in several patients by using human group A red blood cells as the insoluble matrix. The radioallergosorbent test inhibition procedure was done by incubating seminal plasma (30 mg protein/mL) with a seminal plasma-specific serum for 2 hours at 37°C . The incubation mixtures then were assayed by the standard radioallergosorbent test (19). The amount of specific IgE binding (cpm) contained in the absorbed mixture compared to that in the unabsorbed allergic serum $\times 100$ was calculated as the percent radioallergosorbent test inhibition.

Leukocyte Histamine Release: Leukocyte histamine release tests were done by the method of Lichtenstein and Osler (20). Varying concentrations (undiluted to 1:100 000 [w/v]) of spermatozoa extract, unfractionated seminal plasma and seminal plasma fractions were used to conduct a full-dose response assay. In each experiment leukocytes from a normal volunteer served as controls. Histamine release values greater than 25% were considered significant.

Antisperm Antibodies: These were determined by the Kirby-Brick's gelatin agglutination test (13). Both 2-mercaptoethanol sensitive (IgM) and insensitive (IgG) antibodies were evaluated. Sperm antibodies of normal persons are zero or negligible.

MIGRATION INHIBITION TESTS

Leukocytes: Peripheral leukocytes were obtained by sedimentation of 25 mL of blood in a solution containing 250 U of heparin/mL and 5% dextran (21). After two separate washes with 0.83% ammonium chloride and Hanks balanced salt solution, cells were suspended in M-199 containing 10% fetal calf serum. This suspension was aspirated into capillary tubes that were plugged, centrifuged at 400 g, cut at cell fluid interfaces and mounted in tissue culture chambers filled with the suspending medium. To insure uniformity of cell density with capillary tubes, only those packed cells of similar length (1.5 to 3 mm) were selected for the experimental procedures. These chambers then were incubated either with or without various antigens at 37°C and in a 5% CO_2 incubator for 18 to 24 hours (22).

Macrophages: Migration inhibition of guinea pig macrophages was done according to the technique of David and associates (23).

Calculations: The migration areas were measured planimetrically and the percent inhibition of either leukocyte or macrophage migration was determined by the following formula:

$$\% \text{ leukocyte or macrophage migration inhibition} = 1 - \frac{\text{migration of cells in presence of antigen}}{\text{migration of cells without antigen}} \times 100$$

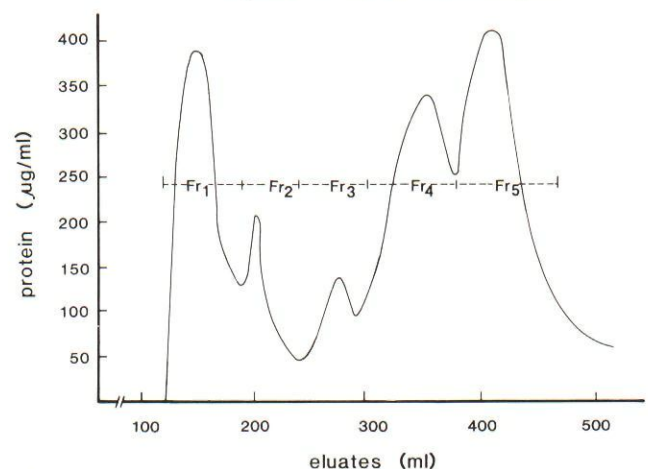


Figure 1. Fractionation pattern of human seminal plasma on Sephadex G-100 chromatography.

Table 2. Immunologic Tests*

Patient	Skin Tests		PT/PCA†		IgE	RAST‡	LHR§		GAT (Sperm)	Lc Prol¶		MIF/LIF**	
	HuSePl	HuSpExt	HuSePl	HuSpExt			HuSePl	HuSpExt		HuSePl	HuSpExt	HuSePl	HuSpExt
					U/mL	← % →							%
1	+(10 ⁻³)††	+(10 ⁻³)	+	+	195	6.6 9.2	48 (2) 44 (3)	27	1:4	ND	ND	0	±
2	+(10 ⁻⁵)	ND	ND	ND	140	0	32 (1)	ND	0	ND	ND	ND	ND
3	+(10 ⁻³)	0	0	0	60	0	ND	ND	1:64	+(12)	+(6)	0	+(20%)
4	0	0	ND	ND	410	0	0	0	ND	0(1.6)	+(4.5)	0	0

* HuSePl = human seminal plasma; HuSpExt = human spermatozoa extract; + = positive; ± = equivocal; 0 = negative; ND = not done.

† PT/PCA = Patient 1, passive transfer (Prausnitz Küstner) tests; Patient 2, passive cutaneous anaphylaxis test.

‡ RAST = radioallergosorbent test of HuSePl in % binding.

§ LHR = % maximum leukocyte histamine release at 1:10 (w/v) antigen dilution. Parentheses indicate the fraction of HuSePl.

|| GAT = gel agglutination test (Patient 1, IgG; Patient 2, IgM and IgG).

¶ Lc Prol = lymphocyte proliferation. Parentheses indicate stimulation index.

** MIF/LIF = Macrophage inhibitory factor/leukocyte inhibitory factor. Parentheses indicate percent inhibition.

†† Parentheses indicate the highest dilution causing a positive skin test.

All experiments were done in duplicate and the mean percent of migration inhibition was calculated. Inhibition percent of 20% or greater was considered to be significant.

Lymphocyte Microcultures: A previously described microculture technique was used for detection of blast transformation (24). Briefly, 60 mL of peripheral blood was collected in a syringe containing 0.4 mL of preservative-free heparin. The blood was diluted 1:2 in Eagle's minimum essential medium containing Earle's salts and L-glutamine. Eight milliliters of the diluted blood was carefully layered onto 4 mL Hypaque-Ficoll and the preparation centrifuged at 300 g for 30 minutes. Mononuclear rich cells were obtained from the gradient interface and suspended in Eagle's minimum essential medium to a concentration of 4×10^6 cells/mL.

Fifty microliters of this cell suspension (2×10^5 cells) were incubated in sterile round plates (Linbro Chemical Co., New Haven, Connecticut) with seminal plasma, seminal plasma fractions and spermatozoa extract. One hundred microliters of minimum essential medium containing 20% heat-inactivated fetal calf serum were added to each plate. The minimum essential medium also contained 10 µg/mL of Gentamycin (Schering Corp., Port Reading, New York), 5 µg/mL of flucytosine (Hoffman-LaRoche, Inc., Nutley, New Jersey), 100 U of penicillin G and 100 µg of streptomycin/mL.

Cultures were maintained at 37 °C, 5% CO₂ tension, for 64 hours, after which 2 Ci of ³H thymidine (Amersham Corp., Arlington Heights, Illinois; [specific activity, 5000 mCi/mmol]) was added. After an 8-hour pulse of the radionucleotide, cultures were harvested with a multiple automated sample harvester (Microbiological Associates, Bethesda, Maryland) whereby cells were collected onto glass-fiber filter strips. After drying, cell containing areas were removed and added to miniature glass scintillation vials. Seven milliliters of a xylene-based, non-aqueous scintillation cocktail were added (Kew Solve, Kew Scientific Inc., Columbus, Ohio). Counting was performed in a beta counter. Each individual experiment was conducted with 10 replicates.

Results of each experiment were expressed as a geometric mean, plus or minus standard errors of the mean of these replicates. A stimulation index was calculated by the ratio of mean uptake of tritiated thymidine of antigen stimulated cultures to that in cultured lymphocytes without antigen.

GENETIC TESTS

Blood group (ABO) typing was done with commercial antisera. Human leukocyte antigen (HLA) typing of each patient and her sexual partner was done using the method of Terasaki and associates (25). The tissue typing plates for the standard microcytotoxicity assay contained antisera directed at a large panel of antigens at the A, B and DRw loci. Specificities of all antisera were previously assured by direct comparison to standard National Institutes of Health antisera. Comparative analy-

sis of gene frequency of HLA-A, -B and DRw loci were based on European caucasoid gene frequency data of the Seventh International Histocompatibility Workshop (1977) and statistical calculations were done according to published methods (26, 27).

Results

Table 2 is a summary of the clinical immunologic results. Intracutaneous and scratch skin tests to both seminal plasma and spermatozoa extract were positive in Patient 1. She also showed direct skin reactivity to seminal plasma Sephadex G-100 fractions 1, 2 and 3 while concurrent tests to her husband's concentrated saliva and serum were negative. The immunologic specificity of immediate skin responses to seminal plasma was confirmed by positive passive cutaneous anaphylaxis procedures in two volunteer recipients (Table 3). Heat treatment or neutralization of the test serum with seminal plasma reduced reaginic activity by 70%. Additional confirmation of an IgE mediated mechanism in this patient was obtained by the radioallergosorbent test done on two separate occasions. The specific binding varied between 6.6% and 9.9%, as compared to a control binding value of 2.8%. A radioallergosorbent test inhibition of 20% was also obtained.

Comparative reaginic potencies of seminal plasma fractions 1, 2 and 3, as determined by passive transfer experiments, are shown in Table 4. Fraction 2 is more potent than either fractions 1 or 3 by factors of 10 and 3, respectively, as determined from the dose response assay of the various challenge antigens used in this experiment. Moreover, the reaginic activity of fraction 2 in this patient is almost twice as high as pooled isogeneic seminal plasma or her husband's seminal plasma.

Nonspecific histamine release (not shown) was observed when either patient's or normal leukocytes were challenged with unfractionated pooled or husband's seminal plasma. However, specific antigen induced leukocyte histamine release was obtained with fractions 2 and 3 of seminal plasma (Table 2).

Specificity of the skin test reactions to spermatozoa extract was also corroborated by passive transfer tests, but not by radioallergosorbent test although the latter is

Table 3. Anaphylactogenic Properties of HuSePl and HuSpExt: Passive Transfer Experiments with Serum of Patient 1*

Challenge Antigens	Protein Concentration	Wheal Areas† of Passive Transfer Sites Treated With:				
		Control Site (Subject 1)	Serum (1:1)		Serum (1:1) + Heated 56 °C 1 hr (Subject 1)	Serum (Undil) + HuSePl (1 mg/mL) (Subject 1)
			Subject 1	Subject 2		
	mg/mL	mm²				
Human Seminal Plasma	1.0	15.9	78.5	67.8	23.7	23.7 (70%)‡
	0.1	ND	12.6	26.7	ND	ND
Human Spermatozoa Extract	1.0	ND	ND	62.3	ND	ND
	0.01	ND	56.7	ND	ND	ND

* HuSePl = human seminal plasma; HuSpExt = human spermatozoa extract; ND = not done; Subjects 1 and 2 are normal volunteer recipients.

† Area was calculated by the formula $A = \frac{\pi d^2}{4}$ where d = mean of long and perpendicular (short) diameters.

‡ Parentheses indicate % neutralization of passively transferable antibody.

known to be less sensitive (28). Spermatozoa extract did not elicit a significant leukocyte histamine release response.

Table 2 shows that weakly positive (1:4), 2-mercaptoethanol insensitive (IgG) anti-sperm agglutinating antibodies were present in the serum of Patient 1. The leukocyte inhibitory factor test was interpreted as equivocal or possibly artifactual because leukocyte migration of both patient and normal daily control was markedly inhibited by all seminal fluid antigens.

Patient 2 had positive scratch and intracutaneous skin tests to dilute solutions of her sexual partner's seminal plasma. She also responded with a positive skin test and significant leukocyte histamine release to fraction 1 of his seminal plasma. Radioallergosorbent test and Kibrick tests were negative. Passive cutaneous anaphylaxis test was not done because a volunteer human recipient could not be obtained.

Apart from an intracutaneous skin reaction to a 1:1000 (w/v) dilution of seminal plasma in Patient 3, IgE-mediated responses were not prominent in patients who presented primarily with local reactions (Patients 3 and 4). Patient 3 manifested unusual Kibrick results. Her vaginal secretions prior to 2-mercaptoethanol treatment gave a Kibrick titer of 1:64 to her husband's sperm. Treatment of her vaginal secretions with 2-mercaptoethanol completely abolished this Kibrick titer. In contrast, the Kibrick titer (1:64) in her serum was completely resistant to the effects of 2-mercaptoethanol. Patient 3 also exhibited positive results of two cell-mediated immunity tests. Her peripheral blood lymphocytes produced a significant amount of the macrophage inhibitory lymphokine after stimulation by spermatozoa extract. In addition, significant blastogenic activity of her lymphocytes occurred after stimulation by both seminal plasma and spermatozoa extract on 2 different test days. Patient 4 did not show any evidence of humoral antibodies. Similar to Patient 3, she was shown to have a positive lymphocyte blast transformation test to spermatozoa extract. However, lymphocyte proliferation did not occur after stimulation with seminal plasma in this case.

The genetic data of all patients and their sexual partners are presented in Table 5. The male sexual partners in Couples 1, 2 and 4 were blood group A positive and

Patients 1 and 3 were O negative. Patient 1 had developed Rh antibodies during her second pregnancy. Histo-compatibility typing of Couples 1 and 3 showed unusual genotypic similarity between the members of each pair. The finding of three identical A-B histocompatibility loci in Couple 1 was highly significant ($p < 0.00001$). The association of identical antigens at two A-B and one DRw loci was also significant ($p = 0.0001$) in Couple 3. Only one common HLA antigen (B 40) was present in Couple 2. In addition, both DRw loci were identical in the partners of the opposite sex of Couples 1 and 2.

Discussion

Cases of adverse reactions to semen have been reported with increasing frequency since the initial presentation on the subject (2). Except for one documented study of localized reaction (12), most of these studies were concerned with systemic symptoms occurring shortly after sexual intercourse. This study was unique because it afforded an opportunity for the relative significance of clinical features and laboratory tests to be compared in four patients by the same investigative team.

These patients were readily classified into two groups defined simply by whether postcoital symptoms were localized or systemic. These clinical categories were further distinguished by the presence of IgE antibodies in the two patients with systemic reactions and positive in vitro correlates of cell-mediated immunity in the two patients with local reactions. IgE antibodies were not detected in the latter group.

When pertinent clinical associations in our patients were analyzed within the context of previously documented cases, several interesting comparisons were evident. Similar to past experience, all but one of our patients were atopic by family and personal history of allergy, as well as positive direct skin tests to common inhalant allergens. Two of our patients (one in each group) experienced their first untoward reactions immediately after their first coital experiences. This unusual phenomenon also occurred in the first reported case of anaphylaxis to seminal fluid (2), and subsequently was also documented in four other patients with localized reactions (12).

Sensitization was postulated to have been caused by

Table 4. Reaginic Potencies of Sephadex G-100 HuSePl* Fractions: Patient 1

Protein Concentration (mg/mL) of Challenge Antigen	Wheal Area† of Passive Transfer Sites Challenged With:				
	P-HuSePl‡	H-HuSePl§	Fraction 1	Fraction 2	Fraction 3
	$\longleftrightarrow$ mm^2 $\longleftrightarrow$				
1.0	153.9	113	70.8	50.2	50.2
0.1	113	44.2	28.3	38.5	44.2
0.01	23.7	28.3	4.9	44.2	15.9

* HuSePl = human seminal plasma.

† Area = $\frac{\pi d^2}{4}$ where d = mean of long and perpendicular diameters.

‡ P-HuSePl = pooled human seminal plasma.

§ H-HuSePl = husband's seminal plasma.

exposure to antigen(s) cross reactive with the antigen(s) of the seminal plasma (2). Other investigators have proposed that naturally occurring anti-sperm antibodies are produced as a response to infection by microorganisms carrying cross-reactivity antigens (29). The current observations in this study are consistent with the hypothesis that prior exposure to cross-reacting antigens (not necessarily microorganisms) could provide the sensitizing stimulus accounting for reactions to seminal fluid on first sexual intercourse.

An interesting temporal relationship of seminal fluid hypersensitivity and the second trimester of pregnancy (one case) or the first coitus after delivery (two cases) had been reported by several other studies (7, 10, 11). One of our patients with systemic manifestations also noted the sudden onset of initial symptoms 6 weeks postpartum. Unfortunately, her IgE-mediated sensitivity is now an ongoing process. Since naturally occurring immunosuppressive agents and enhanced T-cell suppressor activity are known to be present during pregnancy (30-32), it is possible that such a physiologic impairment of immunomodulation, especially if it preferentially involves IgE specific suppressor T-cells, could disappear suddenly after pregnancy and lead to a state of rebound or exaggerated IgE responsiveness to naturally occurring or cross reactive seminal fluid antigenic determinants in genetically susceptible women.

The presence of specific spermatozoal IgE antibodies in one of our patients with immediate hypersensitivity symptoms appears to be the first recorded instance of sperm antigens being identified as anaphylactogenic. Reaginic antibody to her husband's spermatozoa extract was documented by direct skin test, passive cutaneous anaphylaxis and leukocyte histamine release tests (Table 2). Antibodies to spermatozoa extract prepared from a pool of donors was also detected by passive transfer testing.

Specific blastogenesis and migration inhibitory factor responses after stimulation by spermatozoal antigen were observed in two patients who experienced localized reactions. Results of cell-mediated immunity investigations are available in one previous case report, in which allogeneic spermatozoal induced blastogenesis was not considered significant because of the possibility that histocompatibility antigens expressed on sperm membranes might induce a mixed lymphocyte culture response (9). However,

several lines of evidence support the validity of in-vitro cell-mediated responses to spermatozoa extract in our patients (3 and 4). Recent murine experiments revealed that the effects of spermatozoa on the mixed lymphocyte culture reaction were not related to the major histocompatibility locus or associated immune response gene determinants (33). This lack of response may be partially due to the fact that HLA expression on sperm membranes is haploid. Since haploid HLA expression has also been proven for humans (34), spermatozoal antigens might be expected to induce only a weak (if any) blastogenic effect when cultured with allogeneic lymphocytes. Finally, the unusual pattern of the histocompatibility antigenic (two A-B and one DRw) locus sites that Patient 3 shared with her husband would render the possibility of an allogeneic effect less likely in this case.

Similar to all previous reports, seminal plasma was the major anaphylactogen responsible for sensitization in our two patients with systemic reactions. Immunological investigation of this antigen was more complete in Patient 1 (Table 2) in whom the reaginic specificity of seminal plasma was proved by direct skin test, passive cutaneous anaphylaxis, passive cutaneous anaphylaxis neutralization, leukocyte histamine release, radioallergosorbent test and radioallergosorbent inhibition test. The radioallergosorbent test yielded high binding percentages on two different occasions (6.6% and 9.2%), as opposed to the weaker binding values (1% to 3% or grade 1+) previously reported by several different groups of investigators (8, 11). The radioallergosorbent test also ruled out the possibility of IgE mediated response to blood group A substance. Pooled seminal plasma gave as potent skin test and passive cutaneous anaphylaxis results as seminal plasma derived from each patient's sexual partner. The in vitro confirmation of a specific reaginic antibody to seminal plasma was also confirmed by leukocyte histamine release in the second case of seminal fluid anaphylaxis.

Column chromatography (Sephadex G-100) fractions of pooled seminal plasma were also investigated in Patients 1 and 2. Seminal plasma fractions 1, 2 and 3 all reacted briskly at the same concentration of 1×10^{-4} g/mL and therefore could not be differentiated on the basis of direct skin tests. However, passive cutaneous anaphylaxis testing of serum from Patient 1 revealed that the reaginic potency of fraction 2 was greater than the other two fractions and unfractionated seminal plasma itself

Table 5. ABO and Histocompatibility Antigen (HLA) Typing

Couple	ABO	Rh	HLA Genotypes					
1								
Male	A	+	A2	A9	Bw16	B18	DRw1	DRw3*
Female	O	-	<u>A2</u>	<u>A9</u>	<u>Bw16</u>	Bw21	DRw2	DRw5*
2								
Male	A	+	A3	A9	B21	<u>B40</u>	DRw2	DRw5
Female	A	-	A2	A10	B35	<u>B40</u>	DRw1	DRw3
3								
Male	O	+	A10	A29	B14	Bw16	DRw4	DRw7†
Female	O	+	<u>A10</u>	A3	B35	<u>Bw16</u>	<u>DRw4</u>	DRw3†
4								
Male	A	+	A9(Aw24)	A11	B5	B7	DRw2	
Female	A	+	A2	A3	B12	Bw35	DRw4	MT1

* $p < 0.00001$ † $p < 0.0001$

(Table 4). Leukocyte histamine release results in Patient 1 also supported the increased biologic activity of fractions 2 and 3. Fraction 1 apparently was more potent than fractions 2 or 3 in the leukocyte histamine release assay performed in Patient 2 (Table 2).

The most intriguing aspect of this clinical study was the unusual degree of histocompatibility sharing between the members of each of two couples. Since there are no previous reports of histocompatibility typing in women experiencing adverse postcoital reactions, this finding must be interpreted cautiously at the present time. Several investigators have postulated that parental sharing of HLA locus antigens may be associated with a variety of reproductive problems such as fertilization, abortion, gestosis and an enhanced state of maternal tolerance to fetal antigens, as manifested by a decreased response in mixed lymphocyte cultures (35-37). This study suggests that HLA sharing of sexual partners could be a predisposing factor to the subsequent development in women of localized or systemic reactions to components of seminal fluid. The relationship of shared histocompatibility and hypersensitivity reactions to seminal fluid is a useful clinical model for defining aberrant immunologic reactions in the reproductive tract.

The role of IgM and IgG anti-spermatozoal agglutinating antibodies was not clear. These antibodies had only been reported in one other previous case (9), but were found in two of our patients, one in each group. The titers were weak in the systemic reaction but one patient with a local reaction manifested significantly high titers of anti-sperm agglutinating antibodies consisting of both IgM and IgG antibodies.

The pathogenesis of seminal fluid hypersensitivity remains an enigma. Previous investigators have proposed several working hypotheses. The local environment in the female reproductive tract could be altered, increasing the absorption of seminal fluid antigens through the vagina or uterus, or both, in a genetically susceptible host. An alternate suggestion is that normally occurring immunosuppressive factors either in the female reproductive tract or in the seminal plasma may be depressed, thereby causing disruption of the usual immunodulatory processes.

Neither this study nor previous studies have provided a substantive basis for implicating either of these hypotheses. However, the HLA data in this series of patients particularly suggest the contribution of the genetic susceptibility factor in the final expression of clinical disease.

Localized and systemic responses to seminal fluid were completely eliminated by abstinence or use of condoms. The accidental rupture of a condom in Patient 1 on one occasion resulted in a severe anaphylactic episode. Desensitization was not instituted in any of these cases as yet, but the rationale for this form of treatment has been strengthened considerably by a recent report of successful desensitization (11).

The exact incidence of these reactions is unknown. Although formerly investigators believed that their occurrence must be rare, they may be more common than formerly appreciated and may be simply overlooked because of lack of public and physician awareness (11). This, perhaps, is especially true in cases of localized reactions which may mimic other female pelvic inflammatory conditions. Whether local immune responses eventually develop into systemic reactions is not known. Thus far, the two patients with local reactions in this study have been observed for over 3 years without any signs of systemic symptoms. Since reasonably good immunodiagnostic techniques are now available for confirmation of these reactions, future recognition of these problems should be accelerated.

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► Requests for reprints should be addressed to I. Leonard Bernstein, M.D.; University of Cincinnati Medical Center, 231 Bethesda Avenue, Cincinnati, OH 45267.

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